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14. ABSTRACT

Wnt/beta-Catenin signaling and associated target genes are implicated in the establishment of bone metastasis and in the development of castration resistant prostate cancer. Our previous studies have shown that Foxa2 is a Wnt/beta-catenin target gene in prostates. Our preliminary study suggests a Wnt–Foxa2–CXCR4 axis that is involved in PCa bone metastasis, and activation of this axis provides survival mechanisms for PCa cells following androgen deprivation. The hypothesis is that the Wnt/beta-catenin activation of Foxa2 and CXCR4 promotes progression to CRPCa and facilitates bone colonization by PCa cells, and that targeting this axis will provide a novel treatment for PCa bone metastasis and relapse after androgen ablation. In the past one year, our effort mainly focused on addressing if active Wnt/beta-Catenin signaling-induced expression of Foxa2 promotes castration resistant prostate cancer grow in vivo (task 1b); if Foxa2 is involved in the interaction of prostate cancer cells and bone microenvironment (task 2a); and if over-expression of Foxa2 facilitates prostate cancer growth in the bone and progression to castration resistance (task 2b).

15. SUBJECT TERMS
Wnt beta-Catenin, Foxa2, CXCR4, prostate cancer, metastasis, castrate resistant

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Table of Contents

Page

Introduction

Disease-specific mortality in men with prostate cancer (PCa) is almost exclusively the result of the development of castrate resistant (CR) PCa subsequent to hormone ablation. [1] Hormone ablation is the gold standard treatment for metastatic PCa. However, metastasis is the main contributor to mortality in men with advanced PCa. [2]There is no cure for hormone refractory metastatic PCa. Understanding the mechanisms by which PCa cells form metastasis in bone and progress to CRPCa is critical for the development of novel therapeutics. Wnt/β-Catenin signaling and associated target genes are implicated in the establishment of bone metastasis and in the development of CRPCa. High levels of Wnt-1 expression and its down steam mediator, nuclear β-Catenin, are detected in 85% of PCa bone metastasis. We have reported activation of Wnt/beta-Catenin causes HGPIN and enables the prostate to continuously grow following castration,[3, 4] implicating this pathway in the development of castrate resistance. Our studies have also shown that Foxa2, a forkhead transcription factor, is a Wnt/beta-Catenin target gene in the prostate, and that Foxa2 expression is localized at the invasive front in prostate tumors. Our preliminary study confirmed that Foxa2 is expressed in a subset of human PCa bone metastases, implicating Foxa2 in human PCa bone metastasis. My preliminary data indicate that Foxa2 and beta-catenin directly regulate CXCR4 expression, thus establishing the Wnt–Foxa2–CXCR4 axis. We have evidence supporting that the Wnt-Foxa2-CXCR4 axis is involved in PCa bone metastasis, and activation of this axis provides survival mechanisms for PCa cells following androgen deprivation. The hypothesis is that the Wnt/beta-catenin activation of Foxa2 and CXCR4 promotes progression to CRPCa and facilitates bone colonization by PCa cells, and that targeting this axis will provide a novel treatment for PCa bone metastasis and relapse after androgen ablation. This hypothesis will be tested by the following Specific Aims:

Aim 1: To determine if Wnt/β-Catenin signaling induces Foxa2 and CXCR4 to promote CRPCa growth.

Aim 2: To determine if the expression of Foxa2 facilitates castration resistant PCa growth in the bone.

Aim 3: To determine the suitability of pharmacological inhibition of Wnt-Foxa2-CXCR4 axis in conjunction with hormone deprivation to inhibit PCa growth and CR relapse in the bone.

This research will establish the functional implication of the Wnt-Foxa2-CXCR4 axis in PCa progression (metastasis to bone and CR growth). This study will also determine the suitability of this axis as a novel therapeutic target for treating PCa metastasis and relapse after hormone deprivation.

Body

In the past one year, our effort mainly focused on addressing if active Wnt/beta-Catenin signaling-induced expression of Foxa2 promotes castration resistant prostate cancer growth in vivo (task 1b); if Foxa2 is involved in the interaction of prostate cancer cells and bone microenvironment (task 2a); and if over-expression of Foxa2 facilitates prostate cancer growth in the bone and progression to castration resistance (task 2b). We conducted the following research as listed in the statement of work:

Task1b. To determine if active Wnt/beta-Catenin signaling and the expression of FOXA2 promote castration resistant prostate cancer growth *in vivo*.

We proposed to establish CWR22Pc/Foxa2 over-expressing cells in the SOW, but we found out that CWR22Pc is not a pure prostate epithelial cell line, instead, CWR22Pc cells are a mixture of both stromal and epithelial cells, therefore CWR22Pc is not suitable for establishing Foxa2-expressing stable cell line. Therefore, we conducted in vivo experiments using NT1 cells that over-expressing Foxa2. The reason we used NT1 cells for the Foxa2 over-expressing experiments is that NT1 is an AR-expressing but Foxa2-negative prostate epithelial cell line, thus a good model to study how ectopic expression of Foxa2 affects androgen dependency of these cells. We have also established NT1 cells over-expressing a dominant active beta-catenin. We have characterized these cells. Our research found:

1. expression profiles of control NT1, NT1/Foxa2, and NT1/beta-catenin cells

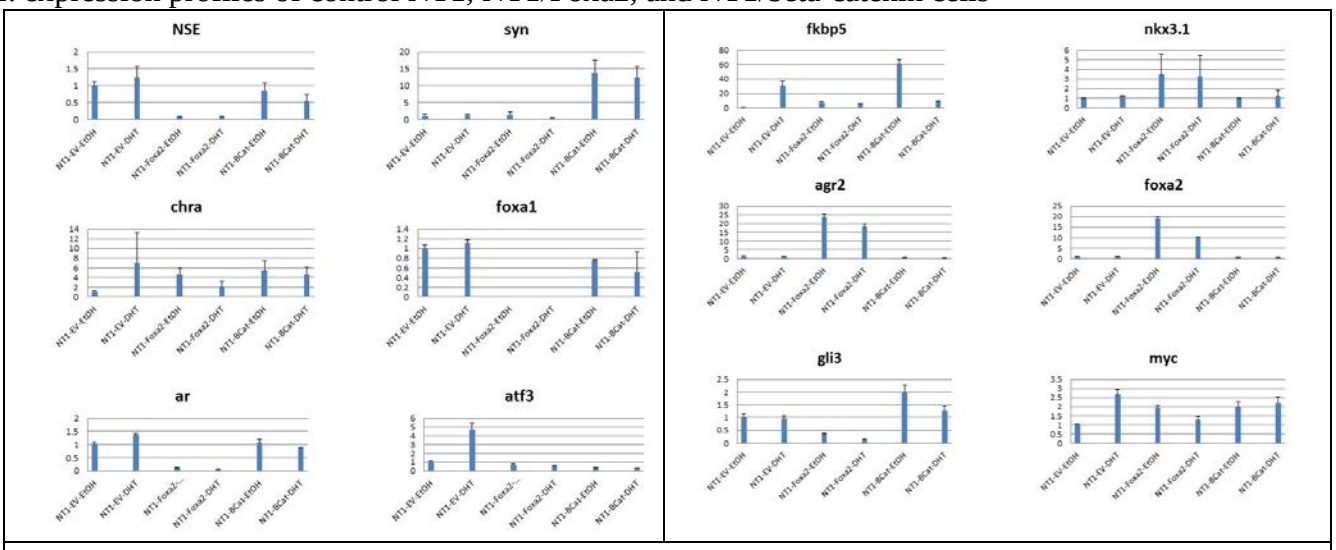

Figure 1. We did RT-PCR to examine the expression of key genes that are involved in prostate cancer progression. We found that the expression of DA beta-catenin turned on neuroendocrine marker synaptophysin, down regulated an AR cofactor ATF3, and up-regulated Gli3, a gene of sonic hedgehog pathway. The expression of Foxa2 down-regulated AR, AR cofactors Foxa1 and ATF3, and AR down-stream target gene FKBP5. Foxa2 also down-regulated Gli3. Agr2, a known Foxa2 target gene, was up regulated in NT1/Foxa2 cells. The down-regulation of Foxa1 by the expression of Foxa2 is particularly interesting because this indicates that Foxa2 might be involved in reprogramming AR transcriptsome since the level of Foxa1 has been implicated in regulating AR selection on target genes.

2. growth of control NT1 and NT/Foxa2 tumors in vivo.

We have also conducted *in vivo* experiments to study the role of Foxa2 in regulating prostate cancer cell growth *in vivo*. Although the expression of Foxa2 enables NT1 cells grow faster *in vitro* (see last year's report), we found, surprisingly, that the NT1/Foxa2 cells grew slower in animal (figure 2A-D), and the expression of Foxa2 did not promote castration-resistant cell growth *in vivo* either, which is different from the *in vitro* results (last year's report).

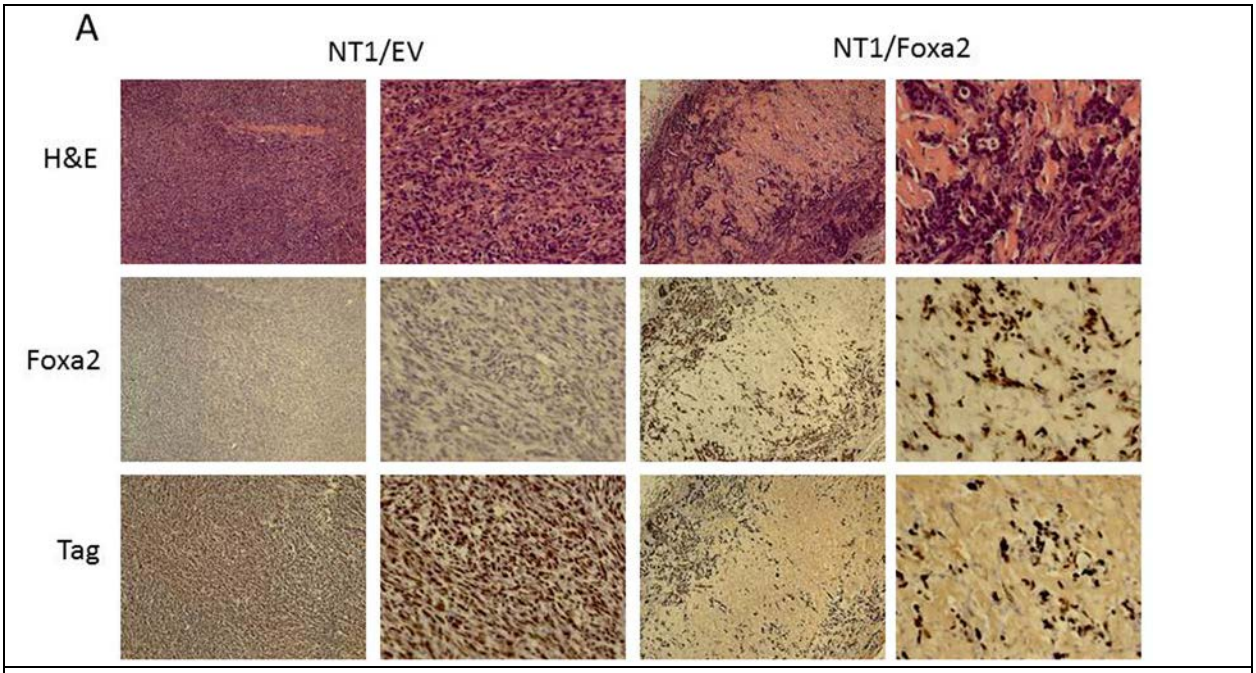

Figure 2A. Histology of control NT1 and NT1/Foxa2 tumors in intact mice. NT1/Foxa2 tumors are smaller and have relative less aggressive histology. The Foxa2 IHC staining confirmed that control NT1 tumors are negative for Foxa2, but NT1/Foxa2 tumors are positive for Foxa2. T-antigen (Tag) staining marked NT1 tumor cells.

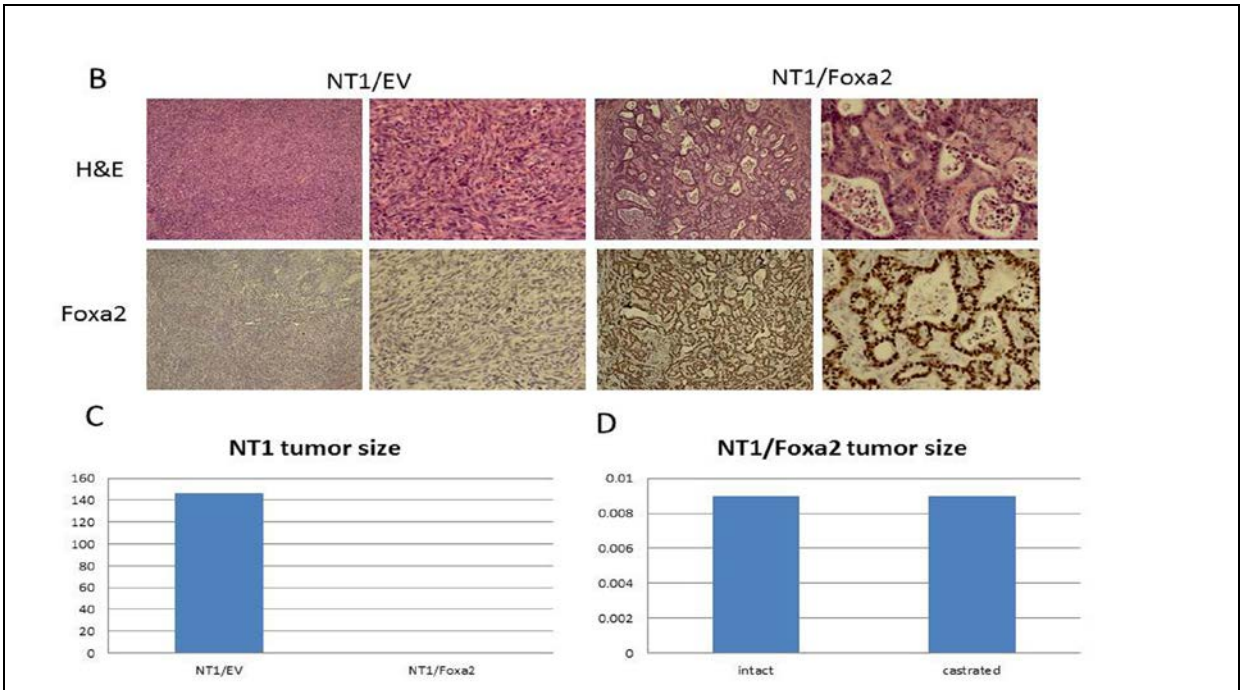

Figure 2B. Histology of control NT1 and NT1/Foxa2 tumors in castrated mice. NT1/Foxa2 tumors displayed less aggressive histology than control NT1 tumors. Figure 2C &2D, the weight of NT1 and NT1/Foxa2 tumors in intact mice (C) and castrated mice (D). NT1/Foxa2 cells formed smaller tumors than the control NT1 cells (C). Castration did not affect NT1/Foxa2 tumor growth. Note: the scale of the x-axis in graph C and D is different.

Task 2a. To determine if FOXA2 is involved in the interaction between prostate cancer cells and the bone microenvironment by controlling the expression of osteoclastogenesis related genes *in vitro*.

We did TRAP staining on monocyte/macrophage lineage progenitor cells treated with conditioned medium collected from NT1 (with or without FOXA2) culture. The multinucleated osteoclasts was visualized by TRAP staining. There is no significant difference resulted from the different treatment.

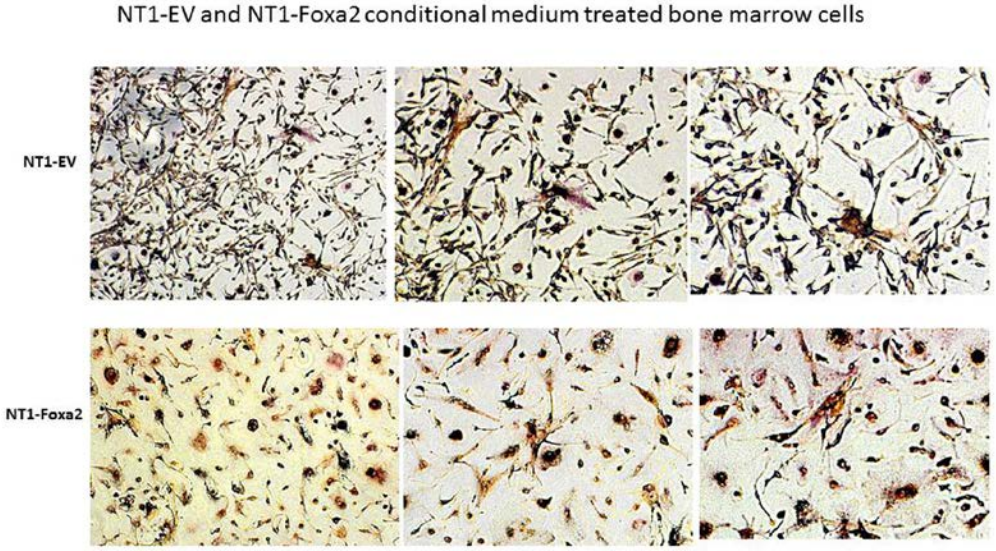

Figure 3. Expression of Foxa2 did not significantly change osteoclastogenesis induced by prostate epithelial cells.

Task 2b. To determine if over-expression of FOXA2 facilitates prostate cancer growth in the bone and progression to castration resistant prostate cancer.

To study the role of Foxa2 in prostate cancer growth in the bone microenvironment, we injected control or Foxa2-expressing NT1 cells into mouse tibias and analyzed the bone lesions mediated by these cells. We found, the expression of Foxa2 reduced bone lesions mediated by NT1 cells (figure 4).

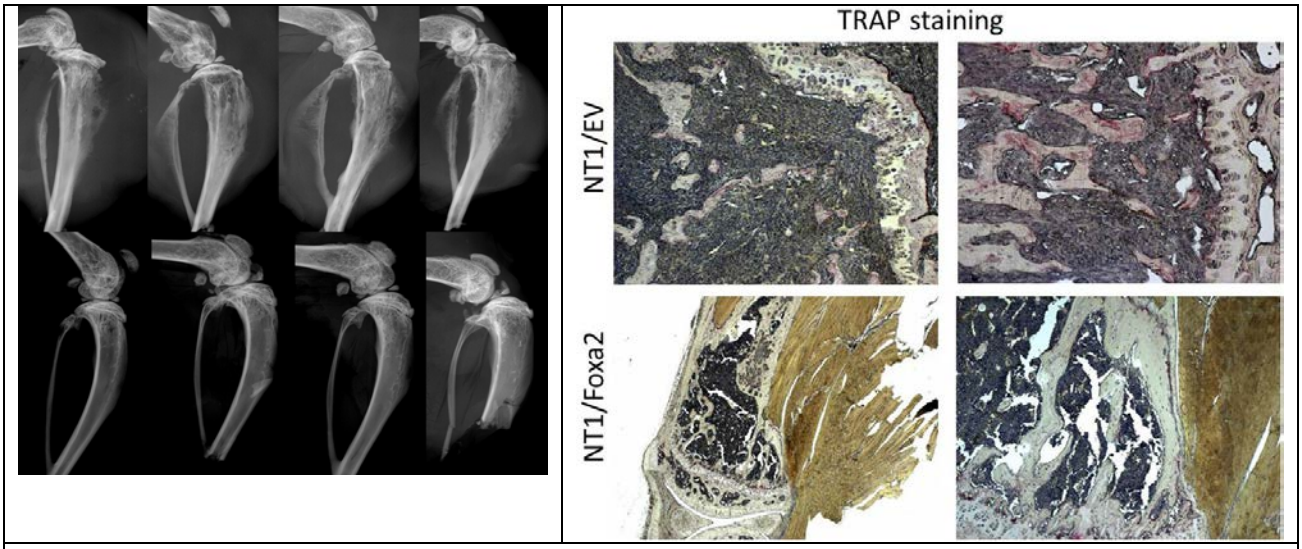

Figure 4. Over-expression of Foxa2 reduced prostate cancer cells-mediated bone lesions. Left: x-ray images showing bone lesions mediated by NT1 cells. Upper images are from control NT1 cell-bearing tibias, lower images from NT1/Foxa2 bearing tibias. While tibias bearing control NT1 cells have severe bone lesions, NT1/Foxa2 cells did not cause as severe bone destruction. Right: TRAP staining performed on sections derived from tibias bearing NT1/EV (upper panels) and NT1/Foxa2 (lower panels) tumors. NT1/EV control tumors caused severe bone destructions while NT2/Foxa2 tumor cells hardly changed the normal histology of tibias.

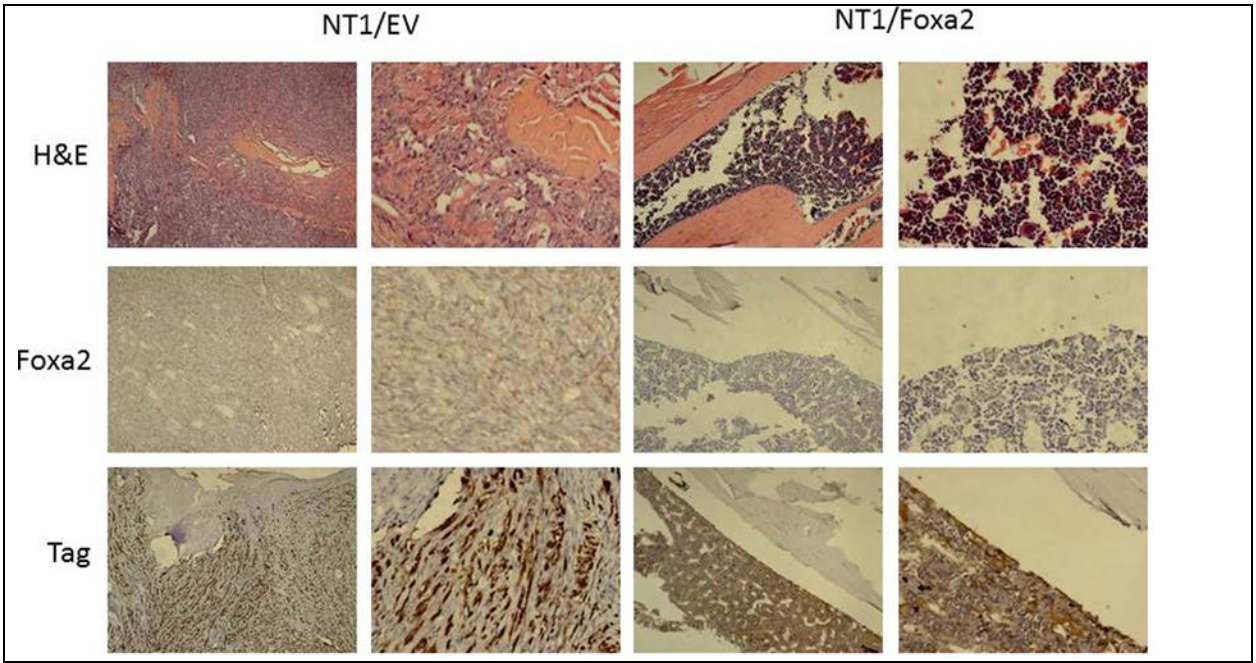

Figure 5. Histological staining of NT1 tumor-bearing tibias. While tibias bearing control NT1 cells have severe bone lesions and tumor growth well in the bone, NT1/Foxa2 cells hardly grow in the bone microenvironment.

key research accomplishments

- We found that the expression of DA beta-catenin turned on neuroendocrine marker and down regulated AR and AR signaling pathway. The expression of Foxa2 also caused down-regulation of AR and AR pathway. A reduction of AR signaling has been shown to accelerate neuroendocrine differentiation in TRAMP mice, suggesting that a down-regulation of AR might be a mechanism how wnt/beta-catenin induces neuroendocrine differentiation, which has become a major research topic in the field now. We will further study this in the future.
- We found the expression of Foxa2 inhibits the expression of Foxa1. Foxa1 is a well established AR cofactor that regulates the expression of differentiation-related genes. The down-regulation of Foxa1 indicates that Foxa2 might be involved in reprogramming AR transcriptsome. This will be another interesting project for us to further pursue.
- Although the expression of Foxa2 enables NT1 cells grow faster *in vitro* (from last year's research), the expression of Foxa2 slowed down NT1 cell growth in vivo. The surprisingly opposite effect observed in these experiments indicates that Foxa2 may co-ordinate with some factors from tumor microenvironment and modulate prostate tumor growth. The opposite results observed in these in vivo and in vitro experiments might be due to the involvement of Foxa2 in regulating angiogenesis in vivo, which was not reflected by the in vitro study.
- Similarly, we found the NT1/Foxa2 cells grow slower than control NT1 cells in the bones.
- And the expression of Foxa2 did not affect NT1 cell-mediated osteoclastogenesis in vitro.

Reportable outcomes

The Sox2 study (see last year's report) was accepted for publication by the journal of "Prostate Cancer and Prostatic Diseases".

Conclusions

1. Activation of Wnt/beta-catenin induces neuroendocrine differentiation.
2. Active Wnt/beta-Catenin and Foxa2 down-regulated AR and AR signaling, indicating that a down-regulation of AR might be the mechanism through which wnt/beta-catenin induces neuroendocrine differentiation.
3. The function of Foxa2 in vivo is surprisingly different from that of in vitro. While the expression of Foxa2 accelerates NT1 cell growth in vitro, it slowed down NT1 cell growth in vivo.
4. The expression of Foxa2 significantly reduced the level of Foxa1, which has been involved in AR reprogramming, suggesting that Foxa2 might change AR transcriptsome and contribute to neuroendocrine differentiation.

References

1. Grossmann ME, Huang H, Tindall DJ. Androgen receptor signaling in androgen-refractory prostate cancer. JNatlCancer Inst 2001; 93(22): 1687-97.
2. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. NatRevMolCell Biol 2003; 4(12): 915-25.
3. Yu X, Wang YQ, Jiang M, et al. Activated beta-catenin in mouse prostate causes HGPIN and continuous prostate growth after castration. The Prostate 2009; 69(3): 249-62.

4. Yu X, Wang Y, DeGraff DJ, Wills ML, Matusik RJ. Wnt/beta-Catenin activation promotes prostate tumor progression in a mouse model. Oncogene 2011; 30(16): 1868-79.
5. Henry MD, Silva MD, Wen S, et al. Spiculated periosteal response induced by intraosseous injection of 22Rv1 prostate cancer cells resembles subset of bone metastases in prostate cancer patients. Prostate 2005; 65(4): 347-54.

Appendice
None